100374

U.S. Environmental Protection Agency
CLP Sample Management Office
209 Madison Street, Alexandria, VA 22313
PHONE: (703) 557-2490 or FTS 557-2490

SAS Number

# SPECIAL ANALYTICAL SERVICES
## Regional Request

☐ Regional Transmittal

☐ Telephone Request

A. EPA Region and Client: EPA Region III

B. Regional Representative: Colleen K. Walling

C. Telephone Number: (301) 266-9180

D. Date of Request:

E. Site Name: Standard Chlorine, Delaware City, De.

Please provide below a description of your request for Special Analytical Services under the Contract Laboratory Program. In order to most efficiently obtain laboratory capability for your request, please address the following considerations, if applicable. Incomplete or erroneous information may result in delay in the processing of your request. Please continue response on additional sheets, or attach supplementary information as needed.

1. General description of analytical service requested:

Analysis of 2 low concentration whole body Fish Samples for pesticides and PCB's. Fish are to be composited and homogenized by the laboratory. Pesticide/PCB extraction and Analysis as per Attachment #1 for TCL pesticides/PCB's. It is Acknowledged only ACID Resistant pesticides will survive the clean-up - Attachment #1.

2. Definition and number of work units involved (specify whether whole samples or fractions; whether organics or inorganics; whether aqueous or soil and sediments; and whether low, medium, or high concentration):

2 low concentration fish samples plus 1 laboratory duplicate plus 1 [redacted] Reagent blank

The awarded laboratory is responsible for meeting all requirements as specified in this client request. Any changes in method(s) or other specifications must be approved by Region III prior to the award. The referenced Statement of Work must be used including all current revisions of that SOW. If these stipulations are not met, Region III will recommend review for reduced payment.

AR304007

3. Program (specify whether Superfund (Remedial or Enforcement), RCRA, NPDES, etc.), Justification for analysis and Site Account Number:
Superfund Enforcement OTJB03NPH6

SAS Approved By:

4. Estimated date(s) of collection:

5. Estimated date(s) and method of shipment:

6. Approximate number of days results required after lab receipt of samples:
DATA package due 35 days after VTSR of last sample

7. Analytical protocol required (attach copy if other than a protocol currently used in this program):
See Attachment #1

8. Special technical instructions (if outside protocol requirements, specify compound names, CAS numbers, detection limits, etc.):
See Attachment #1

9. Analytical results required (if known, specify format for data sheets, QA/QC reports, Chain-of-Custody documentation, etc.). If not completed, format of results will be left to program discretion.
See Attachment #1 And (#12) DATA Requirements

Data package must include: all raw data, all instrument and/or equipment calibration results, calculations, blank results, duplicate results, chain of custody forms, SAS request forms, SAS packing list(s) or traffic report(s), copy of airbill(s), and copies of analyst's logbooks(signed by analyst) with date and time of sample preparation and analysis.

The cover page and all sample report forms MUST be labeled with the complete EPA sample number as it appears on chain of custody and CLP paperwork.

The case narrative must document all problems encountered and the subsequent resolutions. List instrumentation and methods employed for analysis.

11. Name of sampling/shipping contact:

Phone:

12. Data Requirements

| Parameter | Detection Limit | Precision Desired (+ or - Concentration) |
|---|---|---|

All standard, blank, and sample chromatograms and quantitation lists. All calculations must be included. All extraction weights and volumes must be included.

13. QC Requirements

| Audits Required | Frequency of Audits | Limits (Percent or Concentration) |
|---|---|---|

one sample, chosen by the laboratory, is to be analyzed in duplicate. If any target compound (>50% RPD) from the laboratory duplicate, reextraction and reanalysis is required

14. Action Required if Limits are Exceeded

See Attachment #1 and #13) QC requirements, Above

15. Request prepared by: C. Smith

Date: 1/25/90

16. Request reviewed by:

Date:

Please return this request to the Sample Management Office as soon as possible to expedite processing of your request for special analytical services. Should you have any questions or need any assistance, please contact your Regional representative at the Sample Management Office.

ATTACHMENT #4 - PCB Analysis

1. Composite fish samples by grinding. Weigh approximately 10 grams of fish, then grind the 10 gram sample with 30 grams of anhydrous sodium sulfate ($Na_2SO_4$). Allow the mixture to stand until dry and sandy in texture (a minimum of 24 hours). The purpose of this step is to ensure the fish tissue is in intimate contact with the sodium sulfate and moisture has been completely removed from the fish tissue. Do not proceed to the extraction until the tissue has been dried.

2. Transfer the fish tissue and sodium sulfate to a large extraction thimble and cover the mixture with a hexane-rinsed glass wool plug. This will prevent the solids from leaving the extraction thimble and entering the solvent. Perform a soxhlet extraction for a minimum of 16 hours with a minimum volume of 100ml of methylene chloride.

3. Transfer the methylene chloride extract into a 250ml or 500ml Kuderna-Danish apparatus. Boil down the extract on a steambath and perform a solvent exchange into 40ml hexane. Reduce hexane volume to 10ml or less, and clean-up with 2ml concentrated $H_2SO_4$, as per the attached literature reference.

4. Analyze by GC/ECD. Calibration must include 3 concentrations of PCB standards (Arochlors 1242, 1248, 1254 and 1260) and target pesticide from CLP list. The analysis of the extract must be within the standard range or a dilution is required. Blanks carried through the extraction procedure must be analyzed. One of the samples is to be analyzed in duplicate - including duplicate extraction procedure. The last analytical run should be a medium concentration check standard. If the response factor change is >30% of the initial calibration medium concentration standard, all associated samples must be reanalyzed. If blank chromatograms exhibit Any target compound >10% of the low concentration standard, all associated samples must be reextracted and reanalyzed.

5. Second column confirmation of positives >1 ppm required. Calibration and other requirements are the same, except only relevant Arochlor or pesticide standards need be run.

6. Required sample quantitation limit all target compounds of 0.25 (ug/gm) wet weight.

PCBS



*Rapid Silica Gel Cleanup*

Insert small wad of glass wool into Butt tube. Add ca 1 cm layer anhydrous granular $Na_2SO_4$ and 6–7 g silica gel. Tap tube to level layer and wet with $CHCl_3$; then place clean 250-ml stainless steel beaker under Butt tube. To vial containing benzene-acetonitrile solution of sample residue requiring further cleanup, add ca 5 ml $CHCl_3$-acetone (9+1). Transfer solution quantitatively to Butt tube with pipet. Rinse vial with ca 5 ml $CHCl_3$-acetone (9+1) and add to tube. Let solution drain before adding ca 40 ml $CHCl_3$-acetone (9+1). Evaporate solution on steam or water bath and transfer residue to original vial. Repeat TLC development.

## Results and Discussion

The above rapid purification procedure was developed after observing that the major interference in detection of aflatoxins on TLC plates was caused by compounds which were more strongly adsorbed on silica gel than aflatoxins. Aflatoxins were separated from these impurities by adjusting the eluting power of the residue solution to allow the passage of the aflatoxin fraction, but not the impurities, through a short column or plug of silica gel. The purification step was thus reduced to a simple filtration step which can be completed in about 10 min as compared to the AOAC chromatographic purification step requiring more than 90 min. Further reduction in analysis time is accomplished in the liquid extraction step with chloroform. Dilution of the solution (1) rather than evaporation reduces the analysis time by an additional 30 min. With these simplifications, an analysis can be completed in about 75 min as compared to about 2½ hr by the AOAC method.

Table 1 contains results from the AOAC (2) and the modified ferric gel methods. In all cases, total recoveries of aflatoxins were higher by the ferric gel method.

**Table 1. Aflatoxin content of contaminated cottonseed meals as determined by AOAC and modified ferric gel methods**

| Meal | $B_1$, µg/kg | | $B_2$, µg/kg | |
|---|---|---|---|---|
| | AOAC | Fe Gel | AOAC | Fe Gel |
| 4-17 | 234 | 274 | 23 | 32 |
| 3-26 | 363 | 430 | 40 | 25 |
| 1-15 | 414 | 471 | 65 | 55 |
| 3-5 | 325 | 435 | 30 | 30 |
| 1-30 | 125 | 291 | 13 | 40 |
| 22-9 | 332 | 345 | 46 | 29 |
| 3-19 | 216 | 257 | 34 | 35 |
| 3-15 | 229 | 257 | 30 | 30 |
| 4-30 | 252 | 258 | 46 | 65 |
| 3-20 | 225 | 277 | 42 | 46 |
| 22-20 | 61 | 302 | 1 | 7 |
| 6-26 | 57 | 42 | 8 | 6 |
| 3-1 | 23 | 26 | 7 | 5 |

The method described has 2 additional features which are worth stressing. One is that a more effective removal of plant pigments such as carotenoids, chlorophyll, and gossypol is obtained with ferric gel than with lead acetate. The second is that disposal of the hazardous lead salts into water effluent systems is eliminated.

### References

(1) Velasco, J. (1970) *JAOAC* 53, 611–616
(2) *Official Method of Analysis* (1970) 11th Ed., AOAC, Washington, D.C., secs. 26.031–26.039

Received February 23, 1972.
This paper was presented at the AOCS-ISF World Congress, Sept. 28–Oct. 1, 1972, at Chicago, Ill.

# Sulfuric Acid for the Cleanup of Animal Tissues for Analysis of Acid-Stable Chlorinated Hydrocarbon Residues

By [illegible] (Hopkins Marine Station of Stanford University, Pacific Grove, Calif. 93950)

Biological substances interfering with pesticide residue analysis are removed from hexane extracts of animal tissues by shaking them with concentrated $H_2SO_4$. Recoveries are reported for several chlorinated hydrocarbon pesticides and polychlorinated biphenyls (PCB) from fish extracts. This treatment destroys dieldrin and organophosphate pesticides, but affords a rapid, efficient, and economical cleanup of animal tissues for the analysis of DDT, PCB, and other acid-stable chlorinated hydrocarbon residues.

The use of gas-liquid chromatography (GLC) with electron capture detection for the analysis of chlorinated hydrocarbon residues requires the prior separation of these residues from interfering biological substances. Most of the cleanup methods used for this separation employ adsorption column chromatography and constitute the most difficult and time-

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*Reagents*

(a) *Sulfuric*
(b) *Hexane.*
to be suitable
electron captur
(c) *Reference*
bis(*p*-chloro
chloro-2,2-b
1,1-dichloro-
DDD); 2-(*o*-ch
trichloroethane
chlorophenyl)-
chlordane; γ-h
drin; endrin; a
and parathion
Protection Ag
polychlorinated
1254 provided

*Cleanup Proc*

(a) *For add*
ciently cleaned
centrifuge tube
vigorously 30
centrifuge 10
phase by gas c
detection.
(b) *Initial c*
5–10 ml hexan
ously 30 sec
colorless hexa
it is necessary
transfer hexa
concentration.

for PCB in soil take .1-.5 gm dl to 50-100 ml flex follow method here take cleaned extract start EPA meth at 10.5.3.4

consuming step in residue analysis. Some of the problems associated with adsorption column cleanup methods are the involved procedures for adsorbent preparation, the large quantity of glassware required, and the errors that may be introduced by way of adsorptive loss of residues on the column and from interfering substances present on the adsorbent or in the eluting solvent.

Sulfuric acid is used as a cleanup agent in the method of Stanley and LeFavoure (1), who apply acid-impregnated Celite to the cleanup of acid-stable pesticide residues, but their method is subject to many of the problems listed above.

This paper evaluates the rapid, efficient cleanup which results from shaking hexane extracts of animal tissues with concentrated sulfuric acid. This method requires no special preparation of reagents and a minimum of glassware, and normally obviates the need to concentrate extracts before GLC. The application of this cleanup to the analysis of residues in animal tissues is described.

## METHOD

### *Reagents*

(a) *Sulfuric acid.*—Reagent grade.

(b) *Hexane.*—As specified by the manufacturer to be suitable for pesticide residue analysis, using electron capture detection.

(c) *Reference standards.*—1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT); 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD); 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE); 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane (*o,p'*-DDT); 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene (DDMU); γ-chlordane; α-chlordane; γ-hexachlorocyclohexane (lindane); dieldrin; endrin; aldrin; heptachlor epoxide; malathion; and parathion were supplied by Environmental Protection Agency, Perrine, Fla. The mixture of polychlorinated biphenyls (PCB) used was Aroclor® 1254 provided by Monsanto Co., St. Louis, Mo.

### *Cleanup Procedure*

(a) *For additional cleanup.*—Put 10 ml insufficiently cleaned up extract into 15 ml glass-stoppered centrifuge tube, add 1 ml $H_2SO_4$, stopper, and shake vigorously 30 sec. Let phases separate several hr or centrifuge 10 min at ca 600 rpm. Analyze hexane phase by gas chromatography with electron capture detection.

(b) *Initial cleanup.*—Add 1 ml $H_2SO_4$ directly to 8–10 ml hexane extract of tissue and shake vigorously 30 sec. After phases separate, analyze clear, colorless hexane extract by gas chromatography. If it is necessary to concentrate extract to <2 ml, transfer hexane phase to another tube prior to concentration.

## Results and Discussion

Table 1 shows the results obtained when known amounts of pesticides and PCB were added to fish muscle before and after lipid extraction by the method of Stanley and LeFavoure (1). Lipids were extracted with 4 ml hexane/g fish tissue and cleaned up as described. Average recoveries were near 100% for most compounds tested. Aldrin, heptachlor epoxide, and endrin recoveries were approximately 10% lower. Dieldrin, malathion, and parathion were destroyed. PCB components 6 and 7, Fig. 1, could not be resolved in the recovery experiments because of the relatively large DDE content of the samples. It should be noted that the often used extraction technique of Stanley and LeFavoure (3–5), which employs a hot perchloric-acetic acid mixture, destroys several compounds which survive treatment with concentrated sulfuric acid. Of the compounds tested only heptachlor epoxide was affected less by the digestion step than by the cleanup procedure. However, the combination of these methods results in very rapid analyses of those residues which do survive both procedures.

The results of a comparative experiment performed with 10 replicate aliquots of fish lipid extract are presented in Table 2. Each aliquot was equivalent to 521 mg fish tissue. Half of the samples were cleaned up by a method using Florisil column chromatography (6), and the other half were treated with sulfuric acid. The mean values determined for the 4 residues found were 7–15% higher by the acid method than by the Florisil method, and the coefficients of variation by the acid method were virtually the same as the coefficients of variation for replicate GLC analyses of the same extract.

The cleanup method described here is applicable to the analysis of the most persistent of the chlorinated hydrocarbon residues, and its speed, economy, and efficiency make it a valuable tool for uptake experiments or environmental studies which require the analysis of numerous samples.

Sulfuric acid as a cleanup agent was first used in this laboratory for the additional cleanup of sea mammal extracts which had been insufficiently cleaned up by absorptive methods (6–8). Extracts which had resulted in poor chromatograms or which had damaged GLC columns could be analyzed after this treatment. Although the method destroys dieldrin and organophosphorus compounds, Woods and Castle (9) point out that removal of acid-labile pesticides from possible GLC interference can be an advantage in the analysis of DDT and PCB residues.

## Acknowledgments

The author wishes to thank Anne Edwards and James W. Kote for contributing to the development of this method.

Table 1. Recoveries of PCB and pesticides from fish tissue and fish tissue extracts

| Compd Added | Fish Muscle, ppm | Rec. After Addn to Lipid Ext | | Rec. After Addn to Tissue Before Lipid Extn | |
|---|---|---|---|---|---|
| | | Average,[a] % | Range, % | Average,[a] % | Range, % |
| PCB[b] peak 1 | 0.818 | 99 | 97–103 | 101 | 98–106 |
| 2 | 0.817 | 96 | 94–100 | 101 | 96–107 |
| 4 | 0.847 | 99 | 97–100 | 99 | 97–103 |
| 5 | 0.852 | 100 | 97–102 | 98 | 97–100 |
| 9 | 0.852 | 102 | 83–107 | 97 | 93–102 |
| 10 | 0.858 | 102 | 96–107 | 101 | 96–105 |
| 11 | 0.856 | 100 | 94–105 | 95 | 93–95 |
| 12 | 0.825 | 100 | 92–103 | 97 | 92–100 |
| 13 | 0.815 | 100 | 99–101 | 99 | 93–103 |
| 14 | 0.810 | 97 | 94–105 | 102 | 98–107 |
| 15 | 0.808 | 101 | 93–105 | 101 | 98–105 |
| Lindane | 0.850 | 96 | 95–101 | 101 | 100–103 |
| Aldrin | 0.850 | 94 | 91–97 | 11 | 7–17 |
| Heptachlor epoxide | 0.850 | 91 | 88–92 | 93 | 88–91 |
| α-Chlordane | 0.100 | 99 | 98–100 | 98 | 97–100 |
| γ-Chlordane | 0.100 | 99 | 98–99 | 97 | 96–99 |
| DDMU | 0.100 | 99 | 97–100 | 20 | 16–23 |
| *p,p'*-DDE | 0.300 | 99 | 97–100 | 101 | 98–102 |
| *o,p'*-DDT | 0.100 | 99 | 95–101 | 89 | 86–90 |
| *p,p'*-DDD | 0.300 | 98 | 96–100 | 99 | 97–101 |
| *p,p'*-DDT | 0.100 | 98 | 96–101 | 100 | 95–103 |
| Endrin | 0.300 | 94 | 92–97 | 8 | |
| Dieldrin | 0.300 | 4 | 3–6 | | |
| Malathion | 0.200 | 0 | — | | |
| Parathion | 0.200 | 4 | 3–5 | | |

[a] Average of 4 analyses.
[b] 0.500 ppm Aroclor 1254 was added, and the component (Fig. 1) concentrations were estimated from a gas chromatogram of a standard solution by the method of Rote and Murphy (2).
[c] 8 analyses.

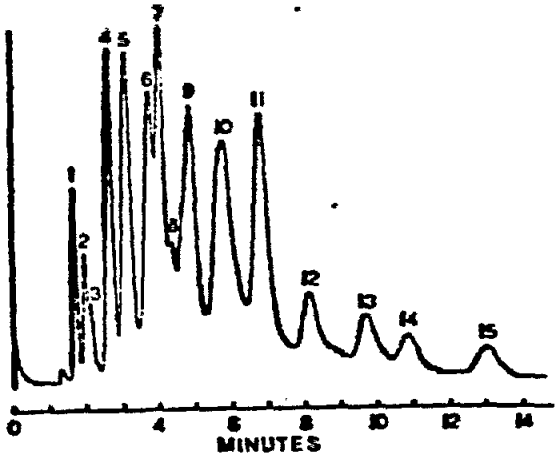


FIG. 1—Gas chromatogram of Aroclor 1254 standard.

Table 2. Residues (ppm) of pesticides in replicate samples of fish lipid cleaned up by 2 methods

| Residue | Acid Method | | Florisil Method | |
|---|---|---|---|---|
| | Mean[a] | C.[b] % | Mean[a] | C.[b] % |
| Lindane | 0.31 | 1.3 | 0.29 | 2.6 |
| *p,p'*-DDE | 0.62 | 1.2 | 0.58 | 3.2 |
| *p,p'*-DDD | 0.32 | 2.0 | 0.34 | 4.6 |
| *p,p'*-DDT | 0.31 | 3.3 | 0.27 | 4.9 |

[a] Mean of 5 analyses.
[b] Coefficient of variation.


## References

(1) Stanley, E. L., & LeFavours, H. T. (1965) *JAOAC* 48, 666–667

(2) Rote, J. W., & Murphy, P. G. (1971) *Bull. Environ. Contam. Toxicol.* 6, 377–384

(3) Woods, L. A., Jr., & Castle, W. T. (1970) *JAOAC* 53, 1304–1305

(4) Risebrough, R. W., Menzel, D. B., Martin, D. S., & Olcott, H. S. (1967) *Nature* 216, 589–591

(5) Cox, J. L. (1970) *Nature* 227, 102–103

(6) *Pesticide Analytical Manual* (1970) Vol. I, Food and Drug Administration, Washington, D.C., sec. HE 212

(7) Kadoum, A. M. (1968) *Bull. Environ. Contam. Toxicol.* 3, 254–259

(8) Cahill, B. J., Fateen, R. J., & Ware, G. W. (1970) *Bull. Environ. Contam. Toxicol.* 5, 70–71


Received February 24, 1972.